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ORIGINAL ARTICLE

Prospective validation of a novel IV busulfan fixed dosing for paediatric patients to improve therapeutic AUC targeting without drug monitoring

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Abstract
Introduction Oral busulfan clearance is age-dependent and children experience a wide variability in plasma exposure. BSA- or age-based dosing is used with therapeutic drug monitoring (TDM) to reduce this variability.
Purpose A new intravenous (IV) dosing of busulfan (Bu) based on body weight, designed to improve AUC targeting without TDM and dose-adjustment, was prospectively evaluated.
Method Bu was administered as a 2 h IV infusion every 6 h over 4 days (16 administrations). Five dose levels were defined on body weight as follows: 1.0 mg/kg for <9 kg; 1.2 mg/kg for 9 to <16 kg; 1.1 mg/kg for 16–23 kg; 0.95 mg/kg for >23–34 kg; 0.80 mg/kg for >34 kg. Bu treatment was followed by Cyclophosphamide or Melphalan prior to allogeneic or autologous transplantation in 55 children aged 0.3–17.2 years (median 5.6 years).
Results No difference in AUC values was observed between weight strata (mean ± SD 1248 ± 205 μmol·min), whereas a significant difference in Bu clearance was demonstrated. This new dosing enabled to achieve a mean exposure comparable to that in adults. At dose 1, 91% of patients achieved the targeted AUC range (900–1500 μmol·min) while no patients were underexposed. At doses 9 and 13, over 75% of patients remained within that target whilst most of the others were slightly above. Successful engraftment was achieved in all patients. In conclusion, from infants to adults this new dosing enabled, without TDM and dose adjustment, to successfully target a therapeutic AUC window.

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Introduction

High-dose busulfan (Bu) is widely used in conditioning regimens as an alternative to total body irradiation prior to haematopoietic stem cell transplantation (HSCT) in adults and children. Considerable knowledge has been acquired for decades through the use of an oral formulation of Bu, the most common dosing for adult patients being 1.0 mg/kg every 6 h for 4 days (16 doses). In Busulfan/Cyclophosphamide (BuCy) conditioning regimens, a range of target area under the drug plasma concentration–time curve (AUC) values, likely to ensure engraftment and reduce severe toxicities, was defined as the therapeutic window. Oral Bu presents a wide inter- and intra-patient variability of plasma exposures, especially in young patients, which could result in poor clinical outcomes [21, 47, 22, 50, 6, 28]. Risk of

hepatic veno-occlusive disease (HVOD) is increased, whereas rates of successful engraftment in allogeneic transplantation setting is decreased when exposures are above or below this therapeutic window. Therefore, controlling patient exposures became a major issue to improve the efficacy and the safety of Bu-based preparative regimens. To optimise Bu treatment, several transplant centres developed Therapeutic Drug Monitoring (TDM) and dose adjustment, in order to enable each patient to reach and to remain within this therapeutic window during the 16-dose treatment. A large experience in adults and adolescents was obtained and a standard and consensual therapeutic window of (900–1500 μmol min) has been defined for BuCy preparative regimens [19, 13, 20, 42, 10, 29, 40].

The use of this therapeutic window was further extended to children receiving a BuCy conditioning regimen. The respective benefit of two approaches, targeted versus non-targeted AUCs, has been evaluated in several non-randomised studies in paediatric patients [45, 26, 5, 30, 4, 41]. They demonstrated a higher rate of successful engraftment, a lower incidence of HVOD and a higher HVOD-free survival with the targeted AUCs approach. However, even if the benefit of this approach is established, the use of TDM is limited because not all investigational centres have facilities for on-line plasma levels measurements and because of the difficulty of collecting blood in infants.

The recently developed intravenous (IV) formulation of Bu provided a more accurate control of both the inter- and the intra-patient variabilities in adults [1, 2, 33, 39], and thus further improved the Bu-based conditioning therapy. This IV formulation was developed as a two-hour infusion in order to achieve comparable C_{max} to the oral pharmacokinetic (PK) profiles, and the dose 0.80 mg/kg IV Bu has been established in adults to result in equivalent AUC values to 1.0 mg/kg oral Bu [1, 34, 3]. A fixed-dose treatment of 0.80 mg/kg IV Bu resulted in 80% of adult patients achieving AUC values within the therapeutic window (900–1500 μmol min) whilst the other 20% were very close to these limits [33]. A reduced incidence of HVOD was observed along with a decreased 100-day mortality rate with IV Bu versus oral Bu, as recently evaluated from clinical outcomes in allo-transplanted adult patients [25, 43, 44, 27].

Controlling Bu exposures in children is more difficult than in adults due to a larger PK variability and to the observed influence of age on the apparent drug clearance, which necessitates the use of different dose levels. Lower exposure, attributed to a higher apparent clearance of oral Bu, has been observed in younger children (≤4 years old) as compared to older children (>4 years old) and to adults [21, 45, 46, 52, 16, 17]. Therefore, higher doses are required in younger children to achieve AUC values comparable to those in older patients. It remains unclear whether the difference in apparent clearance rate observed between the different age groups after oral Bu results from either a true modification in enzyme metabolism activities of the liver [38] (intrinsic clearance) and/or from a modification in the drug absorption process [16, 17]. By skipping the absorption process and escaping drug loss through vomiting, an IV form represents an excellent opportunity to better discriminate the true origin of AUC differences between age groups.

The initial paediatric development of IV Bu (OMC-BUS-5 trial) [51] was carried out using an age-based dosing equivalent to that used with oral Bu (1.2 mg/kg in ≤4 years and 1.0 mg/kg in >4 years). This study confirmed that the difference in plasma exposures in relation to age was due to a higher clearance value in younger than in older children. However, because the IV regimen was designed to mimic the oral regimen practice, TDM was performed on administrations 1, 9 and 13 and doses were adjusted on administrations 5 or 11 based on the target AUC range (900–1,350 μmol min) arbitrarily defined in the protocol. From these results, we hypothesised that age dependency of Bu clearance rate was likely a consequence of a continuous physiological evolution during the child's growth. A retrospective PK analysis based on a population methodology was performed on the OMC-BUS-5 data to further investigate our hypothesis, and to evaluate the relevance of the age cut-off usually used in dose adjustment of oral Bu and of IV Bu. We showed that a continuous log-linear function between Bu clearance and actual body weight (ABW) best explained the age-related variation of IV Bu clearance in children [32]. This modelling allowed us to predict the individual Bu clearance from the child ABW, and therefore to determine the exact individual Bu dose that will enable to achieve the target therapeutic window, as early as the first administration. According to this model, a fixed-dose enabling any individual to reach a pre-defined therapeutic window, as early as the first dose and without drug monitoring or dose adjustment, can be defined. Five fixed dose levels (0.80–1.2 mg/kg) adjusted to five strata of body weights were defined. The potential performance of this new dosing over the usual dosing [i.e. fixed dosing based on either ABW, age, or body surface area (BSA)] was further assessed through population PK simulations. With this new dosing about 75% of simulated patients reached the therapeutic AUC window.

To definitely confirm the benefit of this new dosing in paediatric patients, a prospective clinical trial was performed with a primary objective to demonstrate an efficient AUC targeting into a therapeutic window. Although the evaluation of clinical follow-up is still ongoing, the benefit of this new dosing is already so clearly established that we felt it was important that this knowledge be shared with the transplantation community.

Patients and methods

Study design

The study was a prospective open-label multicentre trial conducted in France between December 2001 and January 2004. The study protocol was approved by the independent ethics committees and the French Health Authority agency. All patients and/or parents/guardian received written information and they all gave written informed consent to treatment in accordance with regulatory and institutional guidelines.

Eligibility criteria included performance status 70% (Lansky or Karnofsky), life expectancy >12 weeks, normal hepatic and renal functions.

Patients from 2 weeks to 18 years old, both genders, were to be enrolled in the study. Patients with solid tumours received the standard Bu and melphalan (Mel) regimen, followed by autologous HSCT as consolidation therapy. Patients with malignant and non-malignant haematological diseases received the standard BuCy regimen followed by allogeneic transplantation as treatment or consolidation therapy.

The IV Bu was infused over 2 h every 6 h for 16 doses. Five fixed IV Bu doses (0.80–1.20 mg/kg) were administered according to five strata of body weights (Table 1). No dose adjustment was allowed during the 4 days of Bu therapy. Following Bu patients received either Cy (50 mg/kg/day × 4 days) or Mel (140 mg/m² for 1 day) after 1 day rest for allogeneic or autologous transplants. Then after 1 day rest (for Cy) patients underwent haematopoietic stem cell transplantation. Benzodiazepine (clonazepam, 0.05–0.1 mg/kg day) as anticonvulsant prophylaxis was administered 12 h before the first Bu administration and until 24 h after the last dose of Bu treatment.

Sampling and Bu determination

Blood samples were obtained following dose 1 (day 1), 9 (day 2) and 13 (day 4), except in the youngest patients (<9 kg) where only 2, 2.5 and 6 h samples were taken. For doses 1 and 9, blood samples were collected at pre-dose, then 1, 2, 2.25, 2.5, 3 and 6 h after the start of infusion. For dose 13, three samples (pre-dose, 2.25 and 6 h) were collected.

Samples were centrifuged and stored at -20°C until bioanalysis. Bu plasma concentration was determined using a gas-chromatography mass-spectrometry (GC–MS) method with a limit of quantification of 62.5 ng/ml [41]. The within- and between-run coefficients of variation were below 10%.

Table 1 Dosage and number of patients

Weight strata (kg)	Dose level (mg/kg)	Nb of patients (Nb of administrations)	Nb of plasma samples (average Nb per patient)
<9	1	8 (24)	93 (11.6)
9 to <16	1.2	14 (42)	218 (15.6)
16–23	1.1	14 (42)	235 (16.8)
>23–34	0.95	6 (18)	102 (17.0)
>34	0.80	13 (39)	219 (16.8)
All strata	0.80–1.2	55 (165)	867 (15.8)

Pharmacokinetic analysis

AUC targeting rate with the new weight-based dosage

Empirical Bayes estimates of clearance and AUC through NONMEM (Version 5.0, post-hoc option) were calculated based on prior information from the covariate-free population model and the individual concentration datasets [38]. Concentration data were fitted by a one-compartment PK model with a first-order elimination and a zero-order input as infusion rate. AUC_{inf} on the first dose and AUC_{ss} at steady state on doses 9 and 13 were calculated from the total administered dose and the estimated individual clearance (Cl_{tot}) values: $AUC_{inf\ or\ ss}$ = total dose/$Cl_{tot.}$

The number of patients to be included (sample size) was determined by the PK primary objective. The one-side chi-square test comparing a theoretical to an observed proportion or frequency was used to calculate the total sample size. The theoretical and expected probabilities and the acceptable error probabilities (Types I and II errors) were as follows: $P_0 = 54\%$; $P_A = 75\%$; $\alpha = 5\%$; $\beta = 20\%$. This assumed that 75% was the minimum desirable probability of success for the new dosage regimen. As a consequence, the planned sample size was 60 evaluable patients (12 per strata of body weight).

The new dosing regimen was rated firstly on the successful targeting performance obtained as early as the first dose, and secondly on the stability of this targeting throughout the treatment period. The number of patients included in the target AUC [(900–1,350 µmol·min), selected in OMC-BUS-5 study] over the total number of patients (P_A = observed probability of success) was calculated and compared to the observed proportion (P_o) obtained from the age-based regimen used in OMC-BUS-5 study [54% of patients included in the target AUC (900–1,350 µmol·min)]. A one-side binomial test ($P_A > P_o$) was used to compare the data. The new dosing strategy was considered better than that based on age if the rate of successful targeting was statistically improved and therefore significantly higher than 54%, taking an α-risk of error of 5%.

For the second assessment of dosing performance the rate of successful targeting was compared between several administrations. The probability of success (number of patients within the targeted AUC over the total number of patients) was determined at doses 9 and 13. The proportion of "discordant" subjects (i.e. success at dose 1 but failure at dose 9 or 13 or vice versa) was compared to 0 by a binomial test at level α of 5% to test the stability of results along the treatment. All the statistical tests were performed using the SAS package program (Version 6.12).

Since a restricted AUC target range (900–1,350 μmol·min) was previously used in OMC-BUS-5 study, the same interval was used in the present study to compare the efficiency of the new dosing regimen to that based on age. However, the risk of developing HVOD if overcoming the AUC upper-limit (1,500 μmol min) is expected to be lower in children than in adults [40, 5, 38, 37]. Therefore, a more realistic therapeutic window (900–1,500 μmol min) was also considered for targeting performances.

Influence of age on PK parameters

Clearance and AUC values were compared between strata of weights using either a two-way ANOVA or an equivalent non-parametric test.

PK parameters were compared between children and adults. Data from adults were from two phase II clinical trials carried out during the clinical development of IV Bu in the USA. Adult patients received 0.80 mg/kg of Bu as a 2 h infusion every 6 h over 4 days (16 doses), followed by cyclosphosphamide (60 mg/kg day-1 × 2). PK results from this adult population were previously reported in a population PK analysis [33].

Consistency of the final population PK model and search for putative new covariate

Predicted clearances based on the previously established [37] body-weight equation [Cl_{tot} (l/h) = 4.57 + 2.97 × [LN (ABW)–3] with ABW in kg] were compared with actual clearances calculated from the full concentration dataset. The final model was appropriate when no major deviations existed between predicted and actual clearances.

Age, height, BSA and gender, hepatic function at baseline (alkaline phosphatases, bilirubinemia and transaminases), type of transplant (autologous versus allogeneic), number of prior cycles of chemotherapy and concomitant anti-fungal therapies were tested as possible covariates likely to influence IV Bu clearance. A NONMEM modelling approach was performed to assess whether new covariates may further influence the Bu PK.

Relationships between Bu exposure and early clinical outcomes

Correlations between Bu exposures and major efficacy/safety early parameters were investigated. Engraftment failure was defined as the inability to reach an ANC ≥ 0.5×10^9/l for three consecutive days by day +100 after allogeneic transplantation. The relationship between Bu AUC and HVOD (Jones criteria [24]) or the worst grade of toxicity (NCI/CTC Version 2.0) recorded in each organ system during the first 28 days post-transplant was explored.

Results

Patients and data

Fifty-five patients ranging 0.3–17.2 years (median = 5.6 years) were included and evaluable: 20 were below the age of 4 years (median = 1.5 years) and 35 above 4 years (median = 8.8 years). All the patients received the full 16 doses IV Bu treatment according to the new dosing strategy, and provided 165 PK profiles (Table 1).

Following IV Bu therapy, 28 and 27 patients were administered IV Cy for allogeneic HSCT and Mel for autologous HSCT, respectively. Demographic and disease characteristics are reported in Table 2.

Evaluation of the dosing to achieve the target AUC

Plasma concentrations versus time data were fitted according to a one-compartment PK covariate-free model. Mean (±SE) population PK parameters were 3.96 ± 0.28 l/h for clearance (Cl_{tot}), and 13.6 ± 1.12 l for volume of distribution (V_c). Inter-patient variability was 55% for Cl_{tot} while intra-patient variability was 9% (Table 4).

Regarding the primary objective on AUC targeting performance, 76.4% of patients AUCs were within the restricted range (900–1,350 μmol min) previously used in OMC-BUS-5 study. The targeting performance was similar in the two groups of patients (78% for autologous and 75% for allogeneic), suggesting that the type of disease has no influence on Bu PK. The mean (±SD) AUC on the whole population (55 patients) at dose 1 was 1,164 ± 180 μmol min (range 824–1,619 μmol min) and therefore very close to the core of the target AUC, i.e. 1,125 μmol min (Fig. 1).

When the more realistic (900–1,500 μmol min) therapeutic window was considered, the successful rate of targeting was 91, 87 and 82% at doses 1, 9 and 13, respectively. No statistically significant period-effect was demonstrated between administrations (NS, Cochran's chi-square tests).

Table 2 Demographic and disease characteristics

Characteristics	Autologous (IV BuMel)	Allogeneic (IV BuCy4)
	27 patients	28 patients
Age (years)		
Median	4.0	7.2
Range	0.7–14.9	0.3–17.2
Weight (kg)		
Median	14.5	27.7
Range	7.7–62.5	5.0–62.0
Gender		
Male	14	15
Female	13	13
Diseases		
Neuroblastoma (NB)	24 (89%)	–
Ewing's sarcoma (EWS)	3 (11%)	–
Acute myeloid leukemia (AML)	–	14 (50%)
Acute lymphocytic leukemia (ALL)	–	1 (4%)
Chronic myelogenous leukemia (CML)	–	3 (11%)
Myelodysplastic syndrome (MDS)	–	1 (4%)
Sickle cell disease (SCD)	–	5 (18%)
Thalassaemia	–	1 (4%)
Wiscott aldrich syndromes (WAS)	–	2 (7%)
Leukocyte adhesion deficiency (LAD)	–	1 (4%)

Individual AUC values over doses 1, 9 and 13 ranged from 847 to 2,088 µmol min with a mean (±SD) of 1,248 ± 205 µmol min. No or very limited under-exposure was observed across administrations: 4 of 165 (2%) evaluated administrations ranged from 847 to 900 µmol min (three patients at dose 1 and one at dose 13). Over-exposure ranging 1,500–2,088 µmol min was observed in 18 (11%) of 165 administrations (two patients at dose 1; seven at dose 9 and nine at dose 13).

Mean Bu clearance values were statistically different ($P < 0.001$, Kruskall–Wallis test) between strata of weight whereas Bu AUC values were not (Table 3, Fig. 2). The comparison of PK data between paediatric ($n = 55$) and adult ($n = 124$) populations further emphasized the interest of this new dosing regimen in children.

Although Bu clearance was 33% higher in paediatric patients than in adults ($P < 0.001$, *t*-test procedure), similar Bu exposures were achieved in both populations. Moreover, the inter-patient variability on Bu AUC values, known to be much higher in children than in adults, was at least similar and even slightly lower in paediatric patients (CV = 16%) than in adults (CV = 19%).

Consistency of the final population PK model and search for putative new covariates

The Bu clearance predicted from the model equation [i.e. Cl_{tot} (l/h) = 4.57 + 2.97 × (LN (ABW)–3) with ABW in kg] was closely correlated with the actual Bu clearance ($R^2 \approx 0.90$) without any significant bias toward the identity line (Fig. 3). Therefore, most of the Bu clearance variation was well predicted by the weight-based model equation, confirming that the model explained most of the inter-patient variability.

When including body weight in the model, the remaining unexplained inter-patient variability (CV) was 17% for Bu clearance, and 14% for volume of distribution. No further fit improvement was achieved when including age, BSA, gender, or other covariates into the final weight-based model. Bu clearance was not affected by prior chemotherapy (at least three previous cycles), or by an increase during the treatment period of either bilirubinemia [up to 8.9 × upper normal limit (UNL)], or alkaline phosphatases (up to 4.2 × UNL) or transaminases (SGPT up to 4.0 × UNL and SGOT up to 2.7 × UNL).

Of note, the current dataset provided parameters in the final model that were consistent with those previously obtained in the OMC-BUS-5 study (Table 4).

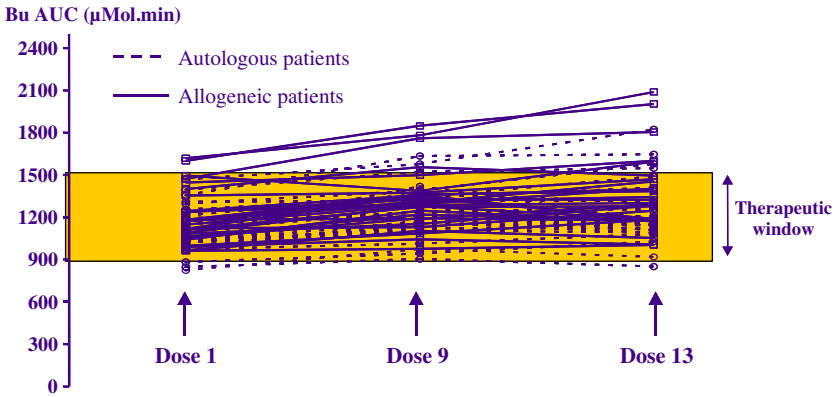

Fig. 1 Bu plasma exposure at doses 1, 9 and 13

Table 3 Statistics of Bu clearance and AUC per strata of weight (doses 1, 9 and 13)

Dose level	Children						Adults [33]
	1 mg/kg	1.2 mg/kg	1.1 mg/kg	0.95 mg/kg	0.8 mg/kg	All 0.8–1.2 mg/kg	0.8 mg/kg
Weight strata	<9 kg	[9–16 kg]	[16–23 kg]	[23–34 kg]	>34 kg	[5–62 kg]	[41–125 kg]
n	8	14	14	6	13	55	127
	IV busulfan clearance (ml/min kg)						
Mean	3.46 ± 0.675	4.06 ± 0.678	3.69 ± 0.571	3.16 ± 0.128	2.57 ± 0.344	3.43 ± 0.762	2.57 ± 0.580
CV (%)	20	17	15	4	13	22	23
Range	2.24–4.50	3.03–5.60	2.47–4.53	2.97–3.27	1.95–3.13	1.95–5.60	1.48–4.60
Statistics	$P < 0.001$					$P < 0.001$	
	IV busulfan AUC (μmol min)						
Mean	1,231 ± 296	1,233 ± 202	1,248 ± 224	1,207 ± 79.0	1,291 ± 182	1,248 ± 205	1,204 ± 233
CV (%)	24	16	18	7	14	16	19
Range	906–1,829	867–1,623	981–1,818	1,088–1,308	1,040–1,679	867–1,829	580–2,199
Statistics	NS					NS	

NS not significant

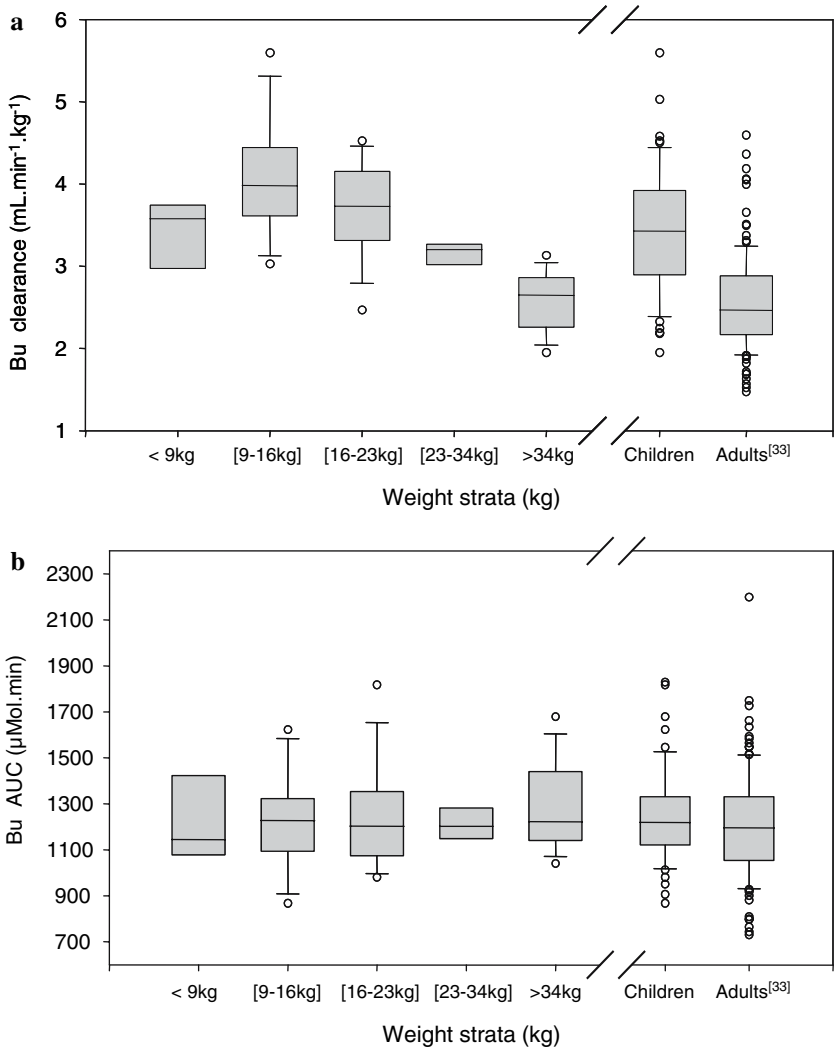

Fig. 2 Bu clearance (a) and Bu AUC (**b**) between strata of weight

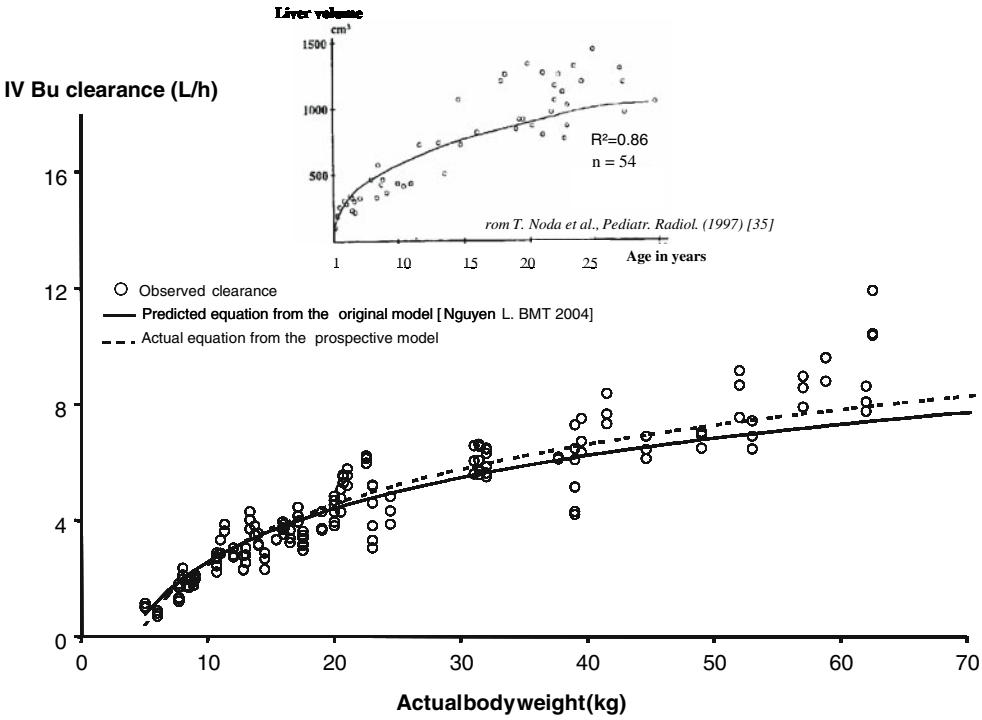

Fig. 3 Relationship between Bu clearance and weight (55 patients and 70 administrations)

Table 4 Population model parameters (RSE in %)

	Predictive equation for clearance (Cl)	Predictive equation for volume of distribution (V_c)	Inter-individual variability in Cl	Inter-individual variability in V_c	Inter-occasion variability in Cl
Prospective model from current study data (n = 55)	Cl (l/h) = $2.62_{(5\%)}$· [LN (ABW)−3] + $4.39_{(3\%)}$	V_c (l) = $ABW^{0.880\ (1\%)}$	17% (22%)	14% (49%)	9% (36%)

Residual variability was modelled with a proportional part (CV = 9.6%) and with an additive part (SD = 60.8 ng/ml)

RSE relative Standard Error of model parameters

ABW actual body weight (kg)

Relationships between Bu exposure and early clinical outcomes

All patients had successful engraftment and therefore PK/PD relationship with graft failures could not be evaluated. HVOD was experienced in 2 (7%) out of 28 allogeneic patients and 4 (15%) of 27 autologous patients. According to McDonald criteria [31] none was severe, four were moderate and two were mild. All resolved in less than 10 days after diagnosis. HVOD incidence was not associated with over-exposure since only one out of six patients with HVOD had an AUC above the upper target limit of 1,500 µmol min (Fig. 4).

In Bu/Mel group, 26/27 patients (96%) experienced one or more episodes of stomatitis: 37% of grade 4, 37% of grade 3 and 22% of grades 1 and 2 according to standard NCI-CTC grading. In BuCy group (n = 28), incidence of severe stomatitis was low: five patients (18%) and one patient (4%) of grades 3 and 4, respectively. PK/PD relationship between stomatitis severity and Bu AUC was demonstrated in Bu/Mel group but not in BuCy group. In the Bu/Mel group, AUC tended to increase with the severity of stomatisis (Fig. 5). Mean AUC in patients with grade 4 stomatitis was significantly higher than that in patients with grades <4 (1,325 ± 178 µmol min; 10 patients, and 1,123 ± 144 µmol min; 17 patients, respectively) (t-test, $P < 0.05$).

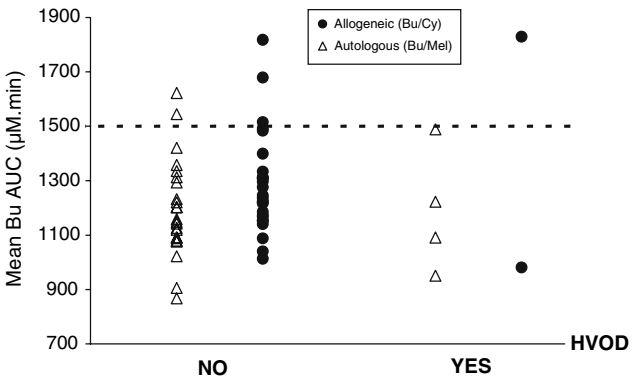

Fig. 4 Individual Bu AUC values in patients with or without HVOD

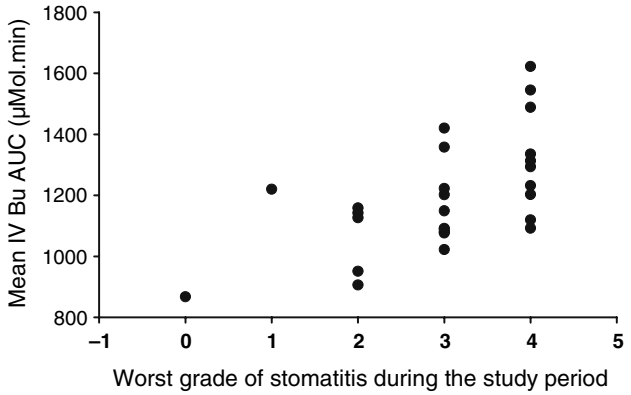

Fig. 5 Mean Bu AUC versus grade of stomatitis in autologous transplants ($n = 27$)

Discussion

Very few anti-cancer agents have been specifically developed for use in children. New medicines are generally extensively evaluated in adults and this knowledge is then unwarrantedly extended to children [48, 14, 15].

Bu is an alkylating agent that has been used orally for more than 40 years at high doses in conditioning regimens prior to stem cell transplantation. Its very poor solubility prevented an easy development of a pharmaceutical intravenous form. The same dosing as that defined in adults was initially administered to children but retrospective analysis illustrated plasma exposures lower (30–50%) in very young children than in older ones [47, 22, 50, 45, 26]. An age threshold close to 4 years was empirically determined and a higher dose level is generally administered in the younger group to achieve exposure similar to that in the older one (1.2 mg/kg instead of 1.0 mg/kg oral Bu). Nevertheless, despite the dose adjustment on age, variability on Bu plasma exposure was still much larger in children than in adults [46, 52, 16, 17].

We previously showed that oral busulfan clearance was higher in young children than in older children and adults, and we suggested that a dose based on BSA (600 mg/m²) may take into account this age-dependent clearance and provide children with the same exposure as adults receiving the usual dosing (16 mg/kg) [46]. Hassan et al. in 1994 [21] used a pioneer formulation of IV Bu extemporaneously prepared, and concluded that Bu clearance normalised to body weight was significantly higher in children ($n = 8$) than in adults ($n = 8$): 3.62 ± 0.78 and 2.49 ± 0.52 ml/min kg, respectively, whereas it became non-significant when normalised to BSA. They also concluded that Bu dosing should rather be calculated on BSA than on body weight, but that TDM and individual dose adjustment should still be considered to avoid drug-related toxicities caused by variability in Bu bioavailability (6-fold in children and 2-fold in adults). More recently, from a larger study conducted on 135 children Gibbs et al. [16] concluded that oral Bu clearance, even when normalised to BSA, was still significantly different between children <4 years and children 8–16 years.

Both our retrospective and prospective analyses of IV Bu data in children indicated that the inter-patient variability of clearance was best explained by a log-linear relationship between absolute clearance and ABW (Fig. 3). As a consequence, the novel dosing strategy was developed on an ABW-based model dosing. PK parameters of the current study (55 patients, 0.3–17.2 years) were comparable to those of the first OMC-BUS-5 study (24 children, 5 months–16.7 years): 3.70 ± 0.43 vs. 3.96 ± 0.28 l/h for Cl_{tot}, and 11.6 ± 1.46 vs. 13.6 ± 1.12 l for V_d, respectively.

This new PK dataset on 55 children with a representative number of patients per category of body weight (from 6 to 14 patients per strata, Table 1), confirmed that the absolute clearance was highly variable between patients (CV = 55%), with marked differences between body weight groups, as expected (Fig. 2a). However, the impact on exposures due to these differences in Cl_{tot} between body weight groups was cancelled out by the different dosing levels administered. As a result, very comparable Bu exposures in children groups were achieved whatever body weight and/or age and, furthermore, exposures were similar to those observed in adults (Fig. 2b and Table 3).

The interest of the new dosing is illustrated from the first dose, since 91% of patients ($n = 50$) were within the therapeutic window (900–1,500 µmol min] versus 67% with the former age-based dosing (76% vs. 58% with the range used in OMC-BUS-5 study (900–1,350 µmol min) ($P < 0.001$). Importantly, the low intra-patient variability (CV = 9%) enabled reproducible AUC within the target for most of the patients. It resulted in an important benefit of the new dosing which enabled to achieve, as early as the first dose and without TDM and dose adjustment, a performance of successful AUC targeting equivalent to that obtained with other dosing strategies that need TDM and dose adjustment. Of note, in the first paediatric study OMC-BUS-5 [51] based on an age-dosing, 22/24 patients (91%) achieved a targeting performance (900–1,500 µmol min) comparable to that obtained in this study, but at dose 9 and after dose adjustment. In Tran' study [45], 18/20 patients (89%) achieved, at steady state (dose 5 or 9) and after dose adjustment up to 1.20 mg/kg, the same AUC target. Of importance, no under-exposure occurred with the new dosing regimen, therefore reducing the risk of graft failure that represents the major risk of Bu conditioning regimen. Under-exposures were frequently observed by several authors when evaluating different dosing strategies and were sometimes still existing after TDM and dose-increase [18, 36, 45, 54].

Concerning the over-exposures observed at dose 13, ranging 1,500–2,080 µmol min, they were limited to 9/55 patients whose baseline characteristics, such as altered hepatic tests, heavy prior chemotherapy or concomitant therapy, do not enable to explain this over-exposure from population PK analysis. Of note, anti-fungal prophylaxis treatment, known to inhibit liver enzyme activities, were concomitantly administered in 12 children (Fluconazole $n = 9$, Itraconazole $n = 1$ and Voriconazole $n = 2$), and there were no differences on Bu plasma exposures between these patients and those who did not receive anti-fungal therapy. From the nine patients presenting an AUC > 1,500 µmol.min at dose 13, no major toxicities occurred; only one patient experienced HVOD, which was moderate. A close correlation between the severity of stomatitis and Bu AUC was established only in children receiving Bu/Mel conditioning regimen (Fig. 4). Gastro-intestinal toxicity correlated with Mel AUC was previously described [7, 49] in children receiving high-dose Mel as a single agent. It was hypothesised that during the period of high-dose Bu treatment, gluthation liver content is depleted and therefore that the toxicity of Mel is increased.

The true origin of the age-related differences in systemic clearance is uncertain. Such influence has been reported for drugs firstly eliminated through hepatic metabolism, and the evolution of liver volume (LV) over age [35] has been considered a major determinant of drug clearance [37]. A non-linear increase of LV over the age (Fig. 3, bottom panel), was described: rapid in infants, slower in children and stable in adolescents. IV Bu drug clearance illustrated a comparable behaviour varying non-linearly with body weight: a sharp increase in infants from origin to about 10 kg, a slower increase up to 40–50 kg and then a stabilisation above 50 kg (Fig. 3). The LV to body weight ratio (cm^3/kg) was described to continuously decrease with age or with body weight [53, 12, 9, 23, 8]. Conversely, an opposite tendency in very young children was demonstrated from the current prospective study and from the retrospective data analysis of OMC-BUS-5 study: the ratio Cl_{tot}/ABW was 3.46 ml/min kg in very young children (<9 kg) compared to 4.06 ml/min kg) in "older" children (9–16 kg) (Table 3). Therefore, a reduced dosing is necessary in infants (1.0 vs. 1.2 mg/kg). This 1.0 mg/kg dosing validated on a relative limited number of infants ($n = 8$) in our study, was supported by another trial carried out in 14 infants aged 0.7–12 months (12 pts: 3.5–7.3 kg; 2 pts: 10 kg) and receiving IV-Bu [11]. Limited over-exposures (one close to 2,000 µmol min) and under-exposures (all >833 µmol min) were observed, with a mean (±SD) Bu exposure at 1,175 ± 317 µmol min. Seventy-nine per cent of infants were within the (900–1,500 µmol min) AUC window.

In conclusion, this study confirmed the benefit of a new IV Bu dosing in children from a prospective validation. The new dosing enabled to efficiently target a well-established AUC window as soon as the first dose and in a better way than with the age-based dosing associated with TDM and dose adjustment. Finally, efficient engraftment was demonstrated and non-haematological toxicity was mild to moderate. The interest of this PK modelling was clearly established on a defined therapeutic window, and can be adapted to any other therapeutic windows according to the type of transplant, disease and depending on the nature and dosage of the combined alkylating agent.

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